highly DC2 selective. Selective clones were DNA sequenced revealing the random peptide insert. A 1-way MLR was performed with varying dilution of each amplified DC2 specific phage to demonstrate the cellular affects on allogeneic T-cell proliferation. **Results:** Plaque assays from the monocyte adsorbed or non-adsorbed linear random peptide library after the three rounds of panning revealed two consensus sequences in 76 of the 78 (97%) isolated clones that were DC2 selective and one sequence found twice (3%) that was non-DC2 selective. The employed circular random peptide library revealed no DC2 selective sequences from 15 isolated in clones from the monocyte adsorbed and non-adsorbed plaque assays. Preliminary MLR data shows a 35 % reduction in allogeneic T-cell proliferation with the DC2 specific phage compared to control phage. **Conclusions:** Data shows that phage display technology can result in isolating highly specific DC2 peptides from a library of 10,000 different phage clones. Preliminary data suggests that binding DC2 specific peptides may inhibit the function of these immunoregulatory cells, leading to enhanced anti-tumor affect of the transplanted donor graft product by shifting it towards an activated Th1 immune response.

GVH/GVL

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TREATMENT WITH GRANULOCYTE COLONY-STIMULATING FACTOR AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION FOR ACUTE LEUKEMIA INCREASES THE RISK OF GRAFT-VERSUS-HOST DISEASE AND DEATH

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Purpose: Granulocyte colony-stimulating factor (G-CSF) is given after bone marrow transplantation (BMT) to shorten the neutropenic phase. Its effects have not been evaluated in a large patient population. **Patients and Methods:** We studied 1789 patients with acute leukaemia receiving BMT and 434 patients receiving peripheral blood stem cells (PBSC) from HLA-identical siblings from 1992 to 2002 and reported the findings to the European Group for Blood and Marrow Transplantation (EBMT). Among the BMT and PBSC patients, 501 (28%) and 175 (40%), respectively, were treated with G-CSF during the first 14 days after the transplant. The outcome variables were entered in a Cox proportional hazard model. **Results:** BMT and PBSC patients treated with G-CSF had a faster engraftment of absolute neutrophils $>0.5 \times 10^9$/l ($p < 0.01$), but platelet engraftment ($>50 \times 10^9$/l) was slower ($p < 0.001$). In the BMT patients, acute graft-versus-host disease (GVHD) grades II-IV was 50 ± 5% (±95% confidence interval) in the G-CSF group vs. 39 ± 3% in the controls (relative risk (RR) 1.33, p = 0.007, in the multivariate analysis). The incidence of chronic GVHD was also increased (RR 0.29, p = 0.03).

G-CSF was associated with an increase in transplant-related mortality (TRM) (RR 1.73, p = 0.00016), had no effect on relapse, but reduced the survival (RR 1.7, $p < 0.0001$) and leukaemia-free survival rates (LFS) (RR 1.55, p = 0.0003). No such effects of G-CSF were seen in patients receiving PBSC. **Conclusion:** After BMT, platelet engraftment was delayed, and GVHD and TRM were increased. Survival and LFS were reduced. This suggests that G-CSF should not be given shortly after BMT.

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SUBEROYLANILIDE HYDROXAMIC ACID REDUCES ACUTE GRAFT-VERSUS-HOST DISEASE AND PRESERVES GRAFT-VERSUS-LEUKEMIA EFFECT BY INHIBITING HISTONE DEACETYLATION

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Pro-inflammatory cytokines and the loss of gastrointestinal tract integrity contribute to acute graft-versus-host disease (GVHD) whereas the donor cytotoxic responses are critical for graft-versus-leukemia (GVL) preservation. Suberoylanilide hydroxamic acid (SAHA) is an anti-tumor agent that inhibits the activity of histone deacetylases (HDAC) and reduces the production of proinflammatory cytokines. Using a well characterized allogeneic murine BMT model B6 (H-2^b) → B6D2F1 (H-$2^{b/d}$) we studied the effects of HDAC inhibition by SAHA on acute GVHD. Recipients were transplanted with $2x10^6$ donor T and $5x10^6$ BM cells after13 GY TBI. Intra-peritoneal injections of 35 mg/kg/day of SAHA from days +3 to day +7 increased histone H3 acetylation in splenocytes harvested 7 days after BMT, confirming the inhibition of HDAC enzymes. SAHA treatment significantly reduced the serum levels of pro-inflammatory cytokines such as TNF-a, IL-1β and IFN-γ ($P < 0.04$) in the allogeneic recipients on day +7 after BMT. Intracytoplasmic staining by flow cytometry and RPA analysis of the host splenocytes on day +7 confirmed the decrease in the cytokine protein and mRNA. SAHA significantly improved the survival ($P < 0.002$) and reduced intestinal damage from GVHD of the allogeneic recipients . However SAHA did not suppress the donor T cell expansion in vivo and the proliferative and cytotoxic responses to host antigens in vitro measured 7 and 14 days after BMT. To test the effect of SAHA on GVL effects, recipients were injected with lethal doses of P815 (H-2^d) tumor cells at the time of BMT. SAHA treatment resulted in significantly improved leukemia-free survival after allogeneic BMT ($P < 0.05$) whereas all the syngeneic BMT recipients of SAHA died of tumor ruling out direct anti-tumor effects of SAHA. Furthermore SAHA increased H3 acetylation in the splenocytes from both the syngeneic and allogeneic leukemic recipients on day +7, confirming that inhibition of HDAC enzymes alone is not sufficient for leukemia free survival in this system. We also tested the effect of SAHA in a second allogeneic BMT model (BALB/c → B6), where it also significantly improved survival ($P < 0.001$) and preserved GVL effects when recipient mice were injected with lethal doses of EL-4 (H-2^b) tumor ($P < 0.04$). We conclude that HDAC inhibition regulates acute GVHD in these models and suggest that this class of pharmacologic agents may provide a novel strategy to reduce GVHD while maintaining the beneficial GVL effects.

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ABROGATION OF THE INTERACTIONS BETWEEN CXCR3 AND ITS LIGANDS MIG AND IP-10 REDUCES THE SEVERITY OF IDIOPATHIC PNEUMONIA SYNDROME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Chemokines are important mediators in the development of Idiopathic Pneumonia Syndrome (IPS), a major cause of mortality after allogeneic (allo) stem cell transplantation (SCT). We hypothesized, that recruitment of donor T cells to the lung is dependent, at least in part, upon interactions between the chemokines MIG and IP-10 and their receptor CXCR3. CXCR3 is expressed on activated T cells; MIG and IP-10 can be induced in various cell types by IFNγ alone or in combination with TNFa or IL-1ß. We tested this hypothesis using an established murine SCT model wherein lethally irradiated bm1 mice receive SCT from either syngeneic (bm1) or allogeneic (B6Ly5.2) donors. MIG and IP-10 BAL levels were significantly elevated in allo recipients compared to syn controls at weeks 1 (MIG: 162.8 ± 37.6 vs 0; IP-10: 41.1 ± 4.2 vs 0 pg/ml) and 4 (MIG: 153.5 ± 41.7 vs 21.6 ± 9.0; IP-10: 202.0 ± 61.1 vs 3.8 ± 0.9 pg/ml) and correlated with the infiltra-

tion of donor T cells into the lung. To examine the effect of these chemokines on T cell recruitment, we treated allogeneic recipients with polyclonal antibodies (Abs) against MIG and IP-10 or control from day 0 until day 28. Lung pathology at day 28 was significantly decreased in the Ab-treated animals and was associated with significantly decreased numbers of total cells, CD8+T cells and TNFa levels in the BAL fluid (table 1). MIG/IP-10 neutralization also resulted in reduced damage to the GI tract by week 1 and 4 (table 1), whereas liver pathology was unaffected. Clinical GVHD scores were also lower in Ab-treated animals at weeks 5 and 6 after SCT and correlated with a reduction in mortality during this time. In a final set of experiments, we used CXCR3-/- mice as allo SCT donors and found that mice receiving CXCR3-/- cells had decreased lung injury compared to allo wild-type controls at week 4 as measured by BAL cellularity (1.2 ± 0.1 vs. 2.5 ± 0.4 mio), CD8+T cells (0.06 ± 0.01 vs. 0.17 ± 0.03 mio) and TNFa levels (9.0 ± 1.8 vs. 33.4 ± 8.0 pg/ml). In conclusion, our data demonstrate, that interactions between the chemokines MIG and IP-10 and their receptor CXCR3 are important for donor T cell recruitment into the lung and the development of IPS. Approaches focusing on the abrogation of these interactions may prove successful in preventing or treating this serious complication after allogeneic SCT.

Table.

	Lung Path.	Total BAL Cells (mio.)	BAL CD8+ Cells (mio.)	BAL TNFα (pg/mL)	Gut Path. Week 1	Gut Path. Week 4
Syn+ctrl	1.4±0.4	0.6±0.04	0.04±0.003	6.9±0.1	8.0±0.6	13.0±1.0
Allo+ctrl	5.3±0.4	1.6±0.38	0.19±0.047	20.8±3.2	23.2±1.6	19.5±2.3
Allo+anti-MIG/IP-10	3.5±0.4*	0.4±0.04*	0.02±0.002*	7.8±3.0*	13.8±1.6*	13.2±1.6 (+)

*$P < .05$; (+) $P = .06$.

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DONOR LEUKOCYTE-PRODUCTION OF RANTES SIGNIFICANTLY CONTRIBUTES TO THE DEVELOPMENT OF IDIOPATHIC PNEUMONIA SYNDROME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Idiopathic pneumonia syndrome (IPS) is a major cause of mortality after allogeneic (allo) stem cell transplantation (SCT). IPS pathophysiology includes the secretion of inflammatory cyto- and chemokines along with the recruitment of donor T cells and monocytes/macrophages. We hypothesized that leukocyte recruitment during IPS is dependent, at least in part, upon interactions between RANTES and its primary receptor CCR5: RANTES is produced predominantly by CD8+ T cells, epithelium, and fibroblasts and promotes CCR5+ T cell and macrophage infiltration to inflammatory sites. Using an established mouse SCT model, lethally irradiated B6DF1 mice received SCT from syngeneic (B6D2F1) or allogeneic (B6) donors. Pulmonary CCR5 mRNA expression was significantly increased from week 1 to week 6 in allo SCT recipients compared to syngeneic controls and correlated with donor effector cell recruitment to the lung and increasing histopathology scores over time. Pulmonary RANTES mRNA expression was significantly increased from week 1 to 6 after allo SCT and correlated with increases in BAL fluid protein levels at weeks 2 (277.7 ± 21.2 vs 18.2 ± 18.2 pg/ml) and 6 (103.3 ± 40.1 vs 0.8 ± 0.8 pg/ml). Furthermore, percentages of RANTES secreting CD4+ and CD8+ T cells were significantly increased in lungs of allo SCT recipients by week 2 after transplant (CD4: 14.8 ± 5.5 vs 0.7 ± 0.2; CD8: 17.2 ± 5.9 vs 0.6 ± 0.2). This suggested that RANTES production from donor effector cells contributes to the evolution of IPS. We next used wild type (wt) or RANTES-/- mice as allo SCT donors. Lung injury after RANTES-/- SCT was significantly reduced by week 6 compared to wt controls and was associated with decreases in total BAL cellularity and in numbers of CD8+ and CD4+ T cells (Table 1). Although survival (wt: 54% vs RANTES-/-: 66%) and clinical GVHD score (wt: 4.8 ± 0.4 vs RANTES-/-: 4.1 ± 0.4) were not different between groups, injury to GI tract and liver was decreased after RANTES-/- SCT (Table 1). We next completed mixing experiments wherein wt or RANTES-/- bone marrow was supplemented with either wt or RANTES-/- T cells and found that RANTES from both cellular sources contributes to IPS. Collectively, these data demonstrate that RANTES production by donor leukocytes is important to the development of lung, intestinal and hepatic damage after allo SCT and suggest that strategies which disrupt RANTES:CCR5 interactions may be helpful in treating or preventing IPS and target organ GVHD.

Table.

Group	Lung Path.	BAL Cellularity (mio.)	BAL CD8+ (mio.)	BAL CD4+ (mio.)	GI Tract Path.	Liver Path.
Syngeneic	0.2 ± 0.1	0.7 ± 0.3	0.01 ± 0.01	0.1 ± 0.1	4.6 ± 0.8	1.6 ± 0.3
Allo wt	5.3 ± 0.6	2.5 ± 0.2	0.7 ± 0.1	0.5 ± 0.1	14.0 ± 0.8	14.1 ± 1.0
Allo RANTES−/−	2.5 ± 0.5*	0.8 ± 0.2*	0.3 ± 0.1*	0.3 ± 0.1	10.6 ± 1.4*	8.6 ± 0.7*

*$P < .05$.

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ANTIBODY RESPONSE TO H-Y MINOR HISTOCOMPATIBILITY ANTIGENS CORRELATES WITH CHRONIC GRAFT VERSUS HOST DISEASE AND DISEASE REMISSION

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H-Y antigens are important targets of graft versus host disease (GVHD) and graft versus leukemia in male patients who receive hematopoietic stem cell transplants from female donors (F → M HSCT). In previous studies, we demonstrated that male patients who receive stem cells from female donors (F → M HSCT) frequently develop antibody responses to H-Y antigen DBY. To determine whether other Y-chromosome encoded proteins elicit B-cell immune responses in F → M HSCT, we developed ELISA for 5 H- Y antigens: DBY, UTY, ZFY, RPS4Y and EIF1AY and their respective X-homologues. Serum samples from 125 HSCT patients and 136 normal donors were tested (Table below). Overall, 51% of male patients with female donors developed antibody to at least 1 H-Y antigen compared to 18% with antibody to any H-X homolog ($p < 0.001$). All H-X responders were also H-Y responders, and the magnitude of each H-Y response was greater than the corresponding H-X response. Normal males and M → M HSCT patients rarely had antibody detected for any H-Y antigen, however 41% of females had antibody detected for at least one H-Y antigen. F → M HSCT serial samples revealed: 1) antibody to H-Y antigens were absent pre-HSCT, 2) DBY antibody developed 4-8 months post-HSCT either concurrently or before UTY, ZFY, and RPS4Y antibodies, and 3) each H-Y antibody response persisted for at least two years post-HSCT. In order to explore the clinical significance of B-cell response to H-Y antigens, we measured 66 well-characterized F → M HSCT patients six months to two years post-HSCT with our novel five H-Y antigens ELISA panel and correlated these results with clinical outcome. The overall incidence of limited or extensive cGVHD in this cohort was 53%. The presence of an antibody response to 1 or more H-Y antigens was highly significantly correlated with the development of cGVHD ($p < 0.001$), and persistent disease remission ($p < 0.001$). Logistic regression models examining donor age, patient age, unrelated donor, HLA-mismatched donor, stem cell source, disease, T cell depletion, or nonmyeloablative conditioning failed to establish association of these risk factors with either cGVHD or H-Y sero-